

AMENDMENTS TO THE CLAIMS

Listing of Claims:

1. (Currently amended) A process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) of n base-pairs having a (+)-strand and a complementary (-)-strand comprising the steps
 - (i) creating a collection of single-stranded fragments of the (+)-strand of the master sequence wherein all members of the collection have the same 5'-terminus and have a deletion in the 3'-terminus such that the collection represents (+)-strands with a length of n-1, n-2, n-3, nucleotides;
 - (ii) introducing at least one universal or degenerate nucleotide at the 3'-terminus of the (+)-strands produced in step (i);
 - (iii) elongating the (+)-strands produced in step (ii) to the full length of the master sequence using the (-)-strand ~~or fragments thereof~~ of the master sequence as a template strand for the elongation; and
 - (iv) synthesizing a (-)-strand by using the (+)-strand produced in step (iii) as a template strand thereby effecting mutations in the (-)-strand at the positions of the previous universal or degenerate nucleotides compared to the master sequence.
2. (Previously presented) The process of claim 1, wherein the collection of single-stranded fragments in step (i) is created by incorporating nucleotide analogs and subsequent cleavage in alkaline or acidic solution.
3. (Previously presented) The process of claim 2, wherein the nucleotide analog is an alpha-phosphothioate nucleotide and oxidative cleavage is achieved by iodine at the phosphothioate bonds.
4. (Previously presented) The process of claim 1, wherein step (ii) comprises the elongation of the collection of single stranded fragments produced in step (i) with universal base or degenerate base by enzymatic or chemical methods.
5. (Previously presented) The process of claim 4, wherein terminal deoxynucleotidyl transferase or DNA polymerases or DNA/RNA ligases are used for elongation.

6. (Previously presented) The process of claim 1, wherein deoxyinosine, 3-nitropyrrole, 5-nitroindole or a nucleotide analog with promiscuous base pairing property is used as a universal nucleotide in step (ii).
7. (Previously presented) The process of claim 1, wherein N⁶-methoxy-2,6-diaminopurine (K), N⁶-methoxy-aminopurine (Z), hydroxylaminopurine (HAP), 2'-deoxyribonucleoside triphosphate (dyTP), 6H,8H-3,4-dihydropyrimidol [4,5-c][1,2] oxazin-7-one (P), N⁴-aminocytidine, N⁴-hydroxy-2'-deoxycytidine, N⁴-methoxy-2'-deoxycytidine, 8-oxodeoxyguanosine triphosphate (8-oxo-G) or a nucleotide analog with promiscuous base pairing property is used as degenerate nucleotide in step (ii).
8. (Previously presented) The process of claim 1, wherein an oligonucleotide of the general formula

$$p(U)_a(N)_b^*(S)_c[TERM]$$

with

p = 5'-phosphate or hydroxy-group or any chemical group capable of forming diester bonds

U = universal or degenerate bases

a = arbitrary integral number from 0 to 10000

N = mixture of four bases (A/T/G/C (standard nucleotides))

b = arbitrary integral number from 0 to 100

* = cleavable group such as phosphothioate bonds in phosphothioate nucleotides

S = standard nucleotide or nucleotide analog

c = arbitrary integral number from 0 to 100

[TERM] = a dye terminator or any group preventing elongation of the oligonucleotide, with the proviso that a+b>0,

is used in step (ii) to introduce universal or degenerate bases to the collection of single-stranded fragments created in step (i).

9. (Previously presented) The process of claim 8, wherein the oligonucleotide is designed in a way that

- (a) stop codons and/or
- (b) amino acids which disrupt secondary structures,

are avoided in the collection of the mutagenized polynucleotide sequences.

10. (Previously presented) The process of claim 8, wherein the oligonucleotide is designed in a way that

- (a) transition mutations or
- (b) transversion mutations,

are effected in the collection of the mutagenized polynucleotide sequences.

11. (Previously presented) The process of claim 8, wherein the single-stranded fragment created in step (i) which is not ligated with the oligonucleotide is removed using exonuclease.

12. (Previously presented) The process of claim 1, wherein the elongation in step (iii) is effected by a PCR reaction.

13. (Previously presented) The process of claim 1, wherein step (iii) comprises the synthesis of a (-)-single stranded plasmid polynucleotide sequence from a double-stranded plasmid harboring the master sequence using a primer which anneals downstream of the (+)-strand of the master sequence, and annealing of this (-)-single stranded-plasmid polynucleotide sequence with the (+)-strand produced in step (ii), and elongation of the (+)-strand.

14. (Previously presented) The process of claim 1, wherein step (iii) comprises the synthesis of a (-)-single-stranded plasmid harboring the master sequence using a primer which anneals downstream of the (+)-strand of the master sequence in the presence of uracil and standard nucleotides and after the elongation of the (+)-strand produced in step (ii), the uracil carrying (-)-single-stranded plasmid is digested with uracil glycosylase.

15. (Previously presented) The process of claim 1, wherein a PCR amplification is used after step (iii) in order to synthesize a (-)-strand complementary to the (+)-strand produced in step (iii), thereby effecting a double-stranded master sequence carrying mutations.